

Figure 1 BLOSUM62 Substitution Matrix

	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W
C	9	- 1	- 1	- 3	0	- 3	- 3	- 3	- 4	- 3	- 3	- 3	- 3	- 1	- 1	- 1	- 1	- 2	- 2	- 2
S	- 1	4	1	- 1	1	0	1	0	0	0	- 1	- 1	0	- 1	- 2	- 2	- 2	- 2	- 2	- 3
T	- 1	1	4	1	- 1	1	0	1	0	0	0	- 1	0	- 1	- 2	- 2	- 2	- 2	- 2	- 3
P	- 3	- 1	1	7	- 1	- 2	- 1	- 1	- 1	- 1	- 2	- 2	- 1	- 2	- 3	- 3	- 2	- 4	- 3	- 4
A	0	1	- 1	- 1	4	0	- 1	- 2	- 1	- 1	- 2	- 1	- 1	- 1	- 1	- 1	- 2	- 2	- 2	- 3
G	- 3	0	1	- 2	0	6	- 2	- 1	- 2	- 2	- 2	- 2	- 2	- 3	- 4	- 4	0	- 3	- 3	- 2
N	- 3	1	0	- 2	- 2	0	6	1	0	0	- 1	0	0	- 2	- 3	- 3	- 3	- 3	- 2	- 4
D	- 3	0	1	- 1	- 2	- 1	1	6	2	0	- 1	- 2	- 1	- 3	- 3	- 4	- 3	- 3	- 3	- 4
E	- 4	0	0	- 1	- 1	- 2	0	2	5	2	0	0	1	- 2	- 3	- 3	- 3	- 3	- 2	- 3
Q	- 3	0	0	- 1	- 1	- 2	0	0	2	5	0	1	1	0	- 3	- 2	- 2	- 3	- 1	- 2
H	- 3	- 1	0	- 2	- 2	- 2	1	1	0	0	8	0	- 1	- 2	- 3	- 3	- 2	- 1	- 2	- 2
R	- 3	- 1	- 1	- 2	- 1	- 2	0	- 2	0	1	0	5	2	- 1	- 3	- 2	- 3	- 3	- 2	- 3
K	- 3	0	0	- 1	- 1	- 2	0	- 1	1	1	- 1	2	5	- 1	- 3	- 2	- 3	- 3	- 2	- 3
M	- 1	- 1	- 1	- 2	- 1	- 3	- 2	- 3	- 2	0	- 2	- 1	- 1	5	1	2	- 2	0	- 1	- 1
I	- 1	- 2	- 2	- 3	- 1	- 4	- 3	- 3	- 3	- 3	- 3	- 3	- 3	1	4	2	1	0	- 1	- 3
L	- 1	- 2	- 2	- 3	- 1	- 4	- 3	- 4	- 3	- 2	- 3	- 2	- 2	2	2	4	3	0	- 1	- 2
V	- 1	- 2	- 2	- 2	0	- 3	- 3	- 3	- 2	- 2	- 3	- 3	- 2	1	3	1	4	- 1	- 1	- 3
F	- 2	- 2	- 2	- 4	- 2	- 3	- 3	- 3	- 3	- 3	- 1	- 3	- 3	0	0	0	- 1	6	3	1
Y	- 2	- 2	- 2	- 3	- 2	- 3	- 2	- 3	- 2	- 1	2	- 2	- 2	- 1	- 1	- 1	- 1	3	7	2
W	- 2	- 3	- 3	- 4	- 3	- 2	- 4	- 4	- 3	- 2	- 2	- 3	- 3	- 1	- 3	- 2	- 3	1	2	1

Figure 2. Blast alignment of Pfu (query 1) against Deep Vent (subject 1)

>gi|2494186|sp|Q51334|DPOL\_PYRSD DNA polymerase (Deep Vent DNA polymerase)  
[Contains: Endonuclease  
PI-PspI (Psp-GDB pol intein)]  
Length = 1312

Score = 816 bits (2109), Expect = 0.0  
Identities = 414/493 (83%), Positives = 459/493 (92%)

Query: 1 MILDVDYITEEGKPVIRLFKKENGKFKIEHRTFRPYIYALLRDDSKIEEVKKITGERHG 60  
MILD DYITE+GKP+IR+FKKENG+FK+E+DR FRPYIYALL+DDS+I+EV+KIT ERHG  
Sbjct: 1 MILDADYITEDGKPIIRIFKKENGFKVEYDRNFRPYIYALLKDDSQIDEVRKITAERHG 60

Query: 61 KIVRIVDVEKVEKKFLGKPITVWKLYLEHPQDVPTIREKVRHAPAVDIFEYDIPFAKRY 120  
KIVRI+D EKV KKFLG+PI VW+LY EHPQDVP IR+K+REH AV+DIFEYDIPFAKRY  
Sbjct: 61 KIVRIIDAELKVRKKFLGRPIEVWRLYFEHPQDVPAIRDKIREHSAVIDIFEYDIPFAKRY 120

Query: 121 LIDKGLIPMEGEEELKILAFDIETLYHEGEEFEGKGPIMISYADENEAKVITWKNIDLPY 180  
LIDKGLIPMEG+EELK+LAFDIETLYHEGEEF KGPIMISYADE EAKVITWK IDLPY  
Sbjct: 121 LIDKGLIPMEGDEELKLLAFDIETLYHEGEEFAKGPIMISYADEEEAKVITWKKIDLPY 180

Query: 181 VEVVSSSEREMIKRFLRIIREKDPDIIVTYNGDSFDFPYLAKRAEKLGIKLTIGRDGSEPK 240  
VEVVSSSEREMIKRFL++IREKDPD+I+TYNGDSFD PYL KRAEKLGIKL +GRDGSEPK  
Sbjct: 181 VEVVSSSEREMIKRFLKVIREKDPDVIITYNGDSFDLPYLVKRAEKLGIKLPLGRDGSEPK 240

Query: 241 MQRIGDMTAVEVKGRIHFDLYHVITRTINLPTYTLEAVYEAIFGKPKKEKVYAIEIAKAW 300  
MQR+GDMTAVE+KGRIHFDLYHVI RTINLPTYTLEAVYEAIFGKPKKEKVYA EIA+AW  
Sbjct: 241 MQRIGDMTAVEIKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKKEKVYAIEIAEAW 300

Query: 301 SGENLERVAKYSMEDAKATYELGKEFLPMEIQLSRLVGQPLWDVSRSSSTGNLVEWFLLRK 360  
+G+ LERVAKYSMEDAK TYELG+EF PME QLSRLVGQPLWDVSRSSSTGNLVEW+LLRK  
Sbjct: 301 TGKGLERVAKYSMEDAKVTYELGREFFPMEAQLSRLVGQPLWDVSRSSSTGNLVEWYLLRK 360

Query: 361 AYERNEVAPNKPSEEEYQRRRESYTGGFVKEPEKGLWENIVYLDLFRALYPSIIITHNVS 420  
AYERNE+APNKP E EY+RRRESY GG+VKEPEKGLWE +V LDFR+LYPSIIITHNVS  
Sbjct: 361 AYERNELAPNKPDEREYERRRESYAGGYVKEPEKGLWEGLVSLDFRSLYPSIIITHNVS 420

Query: 421 PDLNLEGCKNYDIAPQVGHKFCKDIPGFIPSLLGHLLEERQKIKTKMKETQDPIEKILL 480  
PDLN EGC+ YD+AP+VGHKFCKD PGFIPSLL LL+ERQ+IK KMK ++DPIEK +L  
Sbjct: 421 PDLNREGCREYDVAPEVGHKFCKDFPGFIPSLKRLLEDERQEIKRKMKASKDPIEKML 480

Query: 481 DYRQKAIKLLANS 493  
DYRQ+AIK+LANS  
Sbjct: 481 DYRQRAIKILANS 493

Figure 2--continued. Blast alignment of Pfu (query 1) against Deep Vent (subject 1)

Score = 473 bits (1216), Expect = e-133  
Identities = 248/283 (87%), Positives = 269/283 (94%)

Query: 492 NSFYGYGYAKARWYCKECAESVTAWGRKYIELVWKELEEKFGFKVLYIDTDGLYATIPG 551  
NS+YGYGYAKARWYCKECAESVTAWGR+YIE V KELEEKFGFKVLYIDTDGLYATIPG  
Sbjct: 1029 NSYGYGYGYAKARWYCKECAESVTAWGREYIEFVRKELEEKFGFKVLYIDTDGLYATIPG 1088

Query: 552 GESEEEKKKALEFVKYINSLPGLLELEYEGFYKRGFFVTKKRYAVIDEEGKVITRGLEI 611  
+ EEIKKKALEFV YIN+KLPGLLELEYEGFY RGFFVTKK+YA+IDEEGK+ITRGLEI  
Sbjct: 1089 AKPEEIKKKALEFVDYINAKLPGLLELEYEGFYVRGFFVTKKKYALIDEEGKIITRGLEI 1148

Query: 612 VRRDWSEIAKETQARVLETILKHGDVEEAVRIVKEVIQKLANYEIPPEKLAIYEQITRPL 671  
VRRDWSEIAKETQA+VLE ILKHG+VEEAV+IVKEV +KL+ YEIPPEKL IYEQITRPL  
Sbjct: 1149 VRRDWSEIAKETQAKVLEAILKHGNVEEAVKIVKEVTEKLSKYEIPPEKLVIYEQITRPL 1208

Title: METHODS OF MAKING HYBRID PROTEINS  
Inventor: Peter B. Vander Horn. - Filed: Herewith  
Attorney Docket No.: 020130-001420US - Sheet 3 of 20

Query: 672 HEYKAIGPHVAVAKLAAGVKIKPGMVIGYIVLRGDGPISNRAILAEEDPKKHKYDAE 731  
HEYKAIGPHVAVAK+LAA+GVK++PGMVIGYIVLRGDGPIS RAILAEE+D +KKHYDAE  
Sbjct: 1209 HEYKAIGPHVAVAKRLAARGVKVRPGMVIGYIVLRGDGPISKRAILAEEDLRKKHYDAE 1268

Query: 732 YYIENQVLPVAVLRILEGFGYRKEDLRYQKTRQVGLTSLWLNKK 774  
YYIENQVLPVAVLRILE FGYRKEDLR+QKT+Q GLT+WLNKK  
Sbjct: 1269 YYIENQVLPVAVLRILEAFGYRKEDLRWQKTKQTGLTAWLNKK 1311

Figure 3. Assembly of the oligonucleotides into library fragments.

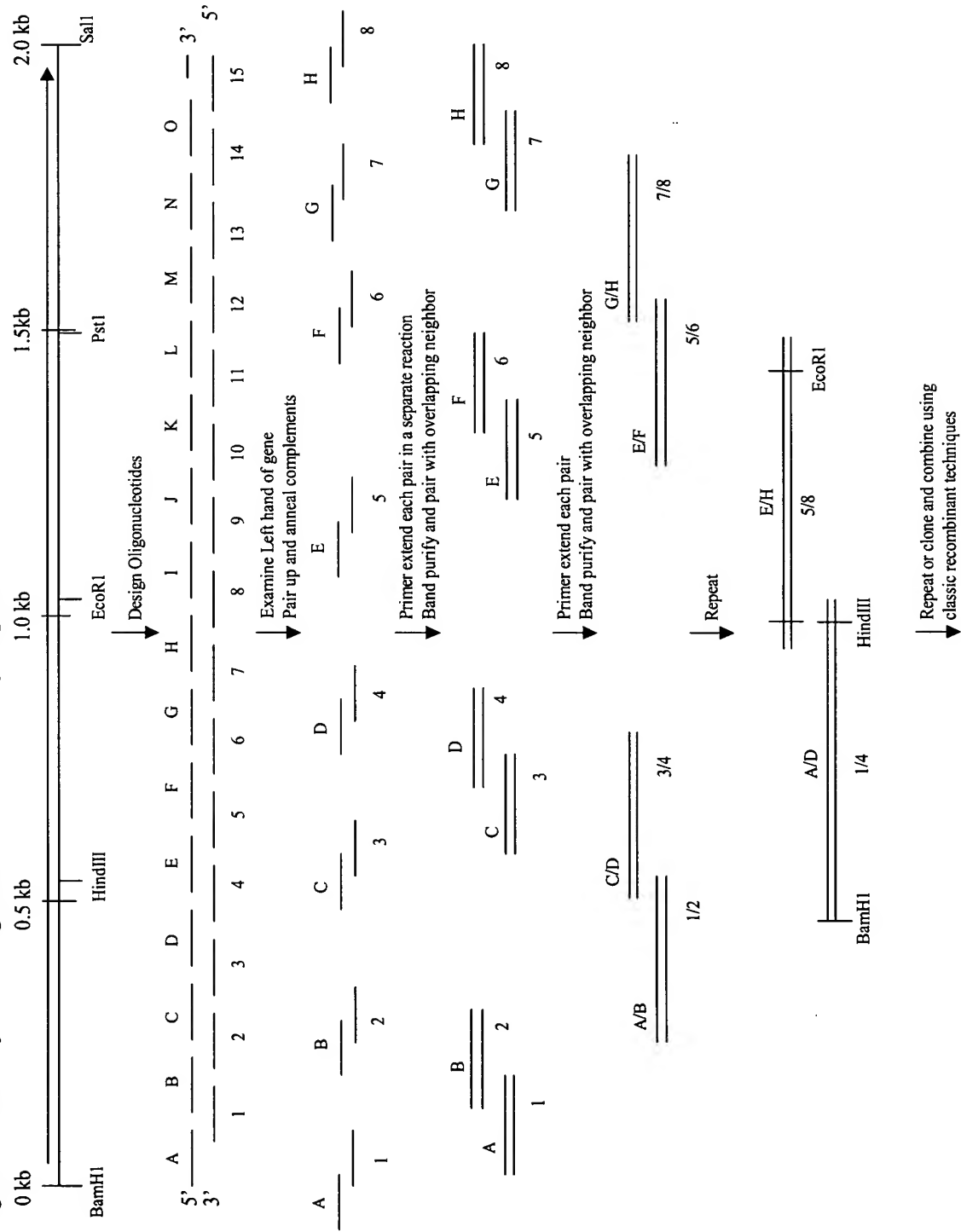


FIG. 4

E coli dut polypeptide sequence:

MKKIDVKILDPRVGKEFPLPTYATSGSAGLDLRACLND AVELAPGDTTLVPTGLAIHIADPSLAAMMLPRSGLGHKHG  
IVLGNLVGLIDSDYQGQLMISVWNRGQDSFTIQGERIAQMIFVPVVQAEFNLVEDFDATDRGEGGFGHSGRQ

AAD polypeptide sequence:

MSKVILKIKRLPHAQDLPLPSYATPHSSGLDLRAAIEKPLKIKPFERVL IPTGLILEIPEGYEQVRPRSGLAWKKGL  
TVLNAPGTIDADYRGEVKVILVNLGNEEVVIERGERIAQLVIAPVQRVEVVEVEEVSQTQRGEGGFGSTGTK

Alignment:

Identities = 61/149 (40%), Positives = 91/149 (60%), Gaps = 1/149 (0%)

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Query: 1  MKKIDVKILDPRVGKEFPLPTYATSGSAGLDLRACLND AVELAPGDTTLVPTGLAIHIAD 60
          M K+ +KI      ++ PLP+YAT  S+GLDLRA +   +++ P +  L+PTGL + I +
Sbjct: 1  MSKVILKIKRLPHAQDLPLPSYATPHSSGLDLRAAIEKPLKIKPFERVL IPTGLILEIPE 60

Query: 61  PSLAAMMLPRSGLGHKHGIVLGNLVGLIDSDYQGQLMISVWNRGQDSFTIQGERIAQMI 120
          + PRSGL  K G+ + N  G ID+DY+G++ + + N G +   I+ GERIAQ++
Sbjct: 61  -GYEQVRPRSGLAWKKGLTVLNAPGTIDADYRGEVKVILVNLGNEEVVIERGERIAQLV 119

Query: 121 FVPVVQAEFNLVEDFDATDRGEGGFGHSG 149
          PV + E   VE+   T RGEggFG +G
Sbjct: 120 IAPVQRVEVVEVEEVSQTQRGEGGFGSTG 148
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**FIGURE 5**  
**5A. Aligned parental sequence showing all possible codons**

ECD	1	M	K	K	I	D	V	K	I	L	D	P	R	V	G	K	E	F	P	L	P	T	Y	A	T	S	G	S	A	G	L	
		ATG	AAA	AAA	ATT	GAT	GTG	AAA	ATT	CTG	GAT	CCG	CGT	GTG	GGC	AAA	GAA	TTT	CCG	CTG	CCG	ACC	TAT	GGC	ACC	AGC	GGC	AGC	GCG	GCG	CTG	
		AAG	AAG	ATC	GAC	GTT	AAG	ATC	TAA	GAC	CCT	CGC	GTT	GGT	AAG	GAG	TTC	CCT	TAA	CCT	ACG	TAC	GCC	ACG	TCT	GGT	TCT	GCC	GGT	TTA	CTA	
				ATA			GTC		ATA	TTC	CCA	CGG	GTC	GGG				CCA	TTG	CCA	ACT		GCA	ACT	AGT	GGG	AGT	GCA	GGG	TTG	CTA	
							GTA			CTT	CCC	CGA	GTA	GGA				CCC	CTT	CCC	ACA		GCT	ACA	TCA	GGA	TCA	GCT	GGA	CTT	CTA	
										CTC			AGA						CTC						TCC	TCC					CTC	CTA
									CTA			AGG							CTA						TCG	TCG					CTA	CTA

AAID	1	M	S	K	V	I	L	K	I	K	R	L	P	H	A	Q	D	L	P	L	P	S	Y	A	T	P	H	S	S	G	L
	ATG	AGC	AAA	GTG	ATT	CTG	AAA	ATT	AAA	CGT	CTG	CCG	CAT	CGC	CAG	GAT	CTG	CCG	CTG	CCG	AGC	TAT	GCG	ACC	CCG	CAT	AGC	AGC	GGC	CTG	
	TCT	AAG	GTT	ATC	TTA	AAG	ATC	AAG	CGC	TTA	CCT	CAC	GCC	CAA	GAC	TTA	CCT	TTA	CCT	TCT	TCT	TAC	GCC	ACG	CCT	CAC	TCT	TCT	GGT	TTA	
	AGT		GTC	ATA	TTG			ATA		CGG	TTG	CCA		GCA		TTG	CCA	TTG	CCA	AGT		GCA	ACT	CCA		AGT	AGT	GGG	TTG		
	TCA		GTA		CTT				CGA	CTT	CCC			GCT		CTT	CCC	CTT	CCC	TCA		GCT	ACA	CCC		TCA	TCA	GGA	CTT		
	TCG				CTC				AGA	CTC						CTC		CTC		TCC						TCC	TCC		CTC		
					CTA				AGG	CTA						CTA		CTA		TCG						TCG	TCG		CTA		

**5B. The minimal encoding sequence**

[illegible]

5C. A minimal encoding sequence after the removal of non-similar degeneracies; selection of ADD parent residues at particular sites

[illegible]

31	D	L	R	A	A	I	E	K	P	L	K	I	K	P	F	E	R	V	L	I	L	E	I	P	E	G
AAD	GAT	CTG	CGT	CGG	CGG	ATT	GAA	AAA	CCG	CTG	AAA	ATT	AAA	CCG	TTT	GAA	CGT	GTG	CTG	ATT	CTG	GAA	ATT	CCG	GAA	GGC
	GAC	TTA	CGC	GCC	GCC	ATC	GAG	AAG	CCT	TTA	AAG	ATC	AAG	CCT	TTC	GAG	CGC	GTT	TTA	ATC	TTA	GAG	ATC	CCT	GAG	GGT
		TTG	CGG	GCA	GCA	ATA			CCA	TTG	ATA			CCA	ACT	GGG	TTG	ATA	TTG			ATA	CCA		GGG	
		CTT	CGA	GCT	GCT				CCC	CTT	CCC			CCC	ACA	GGA	CTT	CTT					CCC		GGA	
		CTC	AGA							CTC	CTC			AGA			AGA	CTC	CTC							
		CTA	AGG							CTA	CTA			AGG			AGG	CTA	CTA							

**5B--continued**

**5C--continued**

GAT	CTG	CGT	CGC	TGC	CTT	AAC	AAT	GCG	GTG	GAA	CTT	GCG	CCG	GGT	GAT	ACG	ACG	CTG	GTT	CCG	ACC	GGC	CTG	ATC	ATT	CAT	ATT	CGC	GAT	GGT
				GC	A	G	G	A	C	A	A	AA	CCG	TTT	A	G	GT		A						C	G	A	C	A	
D	L	R	A	C	L	N	D	A	V	E	L	A	P	G	D	T	T	L	V	P	T	G	L	I	I	H	I	A	D	G
				A	I	E	K	P	L	K	I	K		F	E	R	V		I					I	L	E	P	E		
				S	K	K				E		E		V		M									D					
				G	D	D				T	T	A		C		A									Q					

[illegible]

**5B--continued**

**5C--continued**

TCT	---	GCG	GCG	CAG	ATG	CTG	CCG	CGT	AGC	GGC	CTG	GGC	CTG	GGC	CTG	CTG	AAC	GCG	CTG	CC	GGC	CTG	ATT	GAT	AGC	GAT	TAT			
A		A	G	G	G	G	G	G	C	TGG	AAA	CAT	GGC	ATA	GTG	CTG	CTG	AAC	GCG	CTG	CC	GGC	CTG	ATT	GAT	AGC	GAT	TAT		
S	-	A	A	Q	M	L	P	R	S	G	L	G	W	K	H	G	I	V	L	L	N	A	V	G	L	I	D	S	D	Y
Y		E	G		V	R				A			K	L	T	V				P		P	T			A				
													Q			A				A		A	P			T				
													N			M				L		M				G				



92	R	G	E	V	K	V	I	L	V	N	L	G	N	E	E	V	V	I	E	R	G	E	R	I	A	Q	L	V	I	A	P
AAD	CGT	GGC	GAA	GTG	AAA	GTG	ATT	CTG	GTG	AAC	CTG	GGC	AAC	GAA	GAA	GTG	ATT	GAA	CGT	GGC	GAA	CGT	ATT	GCG	CAG	CTG	GTG	ATT	GCG	CCG	
	CGC	GGT	GAG	GTT	AAG	GTT	ATC	TTA	GTT	AAT	TTA	GGT	AAT	GAG	GAG	GTT	GTT	ATC	GAG	CGC	GGT	GAG	CGC	ATC	GCC	CAA	TTA	GTT	ATC	GCC	CCT
	CGG	GGG	GTC	GTC	GTC	TTG	GTC	TTG	GTC	GGG	TTG	GGG				GTC	GTC	ATA	CGG	GGG	CGG	GGG	CGG	ATA	GCA	TTG	GTC	ATA	GCA	CCA	
	CGA	GGA	GTA	GTA	GTA	CTT	GTA	CTT	GTA		CTT					GGA	GTA		CGA	GGA			GCT	GCT	CTT	GTA	CTT	GTA	GCT	CCC	
	AGA			CTC		CTC		CTC						AGA				AGA								CTC					
	AAG			CTA		CTA		CTA						AGG				AGG								CTA					

**5B--continued**

[illegible]

**5C--continued**

[illegible]

**5B--continued**

5C--continued

[illegible]

FIGURE 6

**KpnI HindIII NdeI**

1 TT GGTACC AAGCTT CAT ATG A(A/G) (A/C) AAA (A/G)TT (G/A) (A/T)T (G/C)TG AAA 38  
 AA CCATGG TTCGAA GTA TAC T(T/C) (T/G) TTT (T/C)AA (C/T) (T/A)A (C/G)AC TTT  
 M K/S/R/N K I/V D/I/V/N V/L K

39 ATT (C/A) (T/A)G CGT C(C/T)G C(G/C)T CAT G(G/C)C (A/C)AA GA(A/T) TT(T/A) 69  
 TAA (G/T) (A/T)C GCA G(G/A)C G(C/G)A GTA C(C/G)G (T/G)TT CT(T/A) AA(A/T)  
 I L/K/Q/M R P/L R/P H G/A K/Q E/D F/L

**StuI**

70 CCG CTG CCG A(C/G)C TAT GCG ACC (T/C)CT CAC AGC (G/T)CA GGC CTG GAT CTG 113  
 GGC GAC GGC T(G/C)G ATA CGC TGG (A/G)GA GTG TCG (C/A)GT CCG GAC CTA GAC  
 P L P T/S Y A T S/P H S A/S G L D L

114 CGT GCG (T/G) (G/C)C (C/A)TT (A/G)A(C/G) (A/G)A(T/A) (G/C)CG (G/C)TG (G/A)AA 140  
 GCA CGC (A/C) (C/G)G (G/T)AA (T/C)T(G/C) (T/G)T(A/T) (C/G)GC (C/G)AC (C/T)TT  
 R A C/A/S/G L/I N/E/K/D D/K/N/E A/P V/L E/K

141 (C/A)TT (G/A) (C/A)G CCG (G/T) (G/T)T GA(T/A) A(C/G)G (A/G) (C/T)G CTG 164  
 (G/T)AA (C/T) (G/T)C GGC (C/A) (C/A)A CT(A/T) T(G/C)C (T/C) (G/A)C GAC  
 L/I A/K/E/T P G/F/V/C D/E T/R T/V/M/A L

**AgeI**

165 (G/A)TT CCG ACC GGT CTG ATC (A/C)TT (C/G)A(T/A) ATT (G/C)CG GA(T/A) GGT 200  
 (C/T)AA GGC TGG CCA GAC TAG (T/G)AA (G/C)T(A/T) TAA (C/G)GC CT(A/T) CCA  
 V/I P T G L I I/L H/E/D/Q I A/P D/E G

201 T(C/A)T G(C/A)G G(C/G)G CAG (A/G)TG C(T/G)G CCG CGT AGC GGC CTG G(G/C)C 236  
 A(G/T)A C(G/T)C C(G/C)C GTC (T/C)AC G(A/C)C GGC GCA TCG CCG GAC C(C/G)G  
 S/Y A/E A/G Q M/V L/R P R S G L G/A

237 TGG AAA (C/A)A(T/G) GGC (A/T)TA (G/A) (T/C)G (C/G)TG CTG AAC GCG 266  
 ACC TTT (G/T)T(A/C) CCG (T/A)AT (C/T) (A/G)C (G/C)AC GAC TTG CGC  
 W K H/K/Q/N G I/L V/T/A/M L/V L N A

**ClaI**

267 (G/C) (T/C)G GGC (C/A) (T/C)G ATC GAT (A/G) (G/C)C GAT TAT C(A/G)G GGC 296  
 (C/G) (A/G)C CCG (G/T) (A/G)C TAG CTA (T/C) (C/G)G CTA ATA G(T/C)C CCG  
 V/P/A/L G L/T/P/M I D S/A/T/G D Y Q/R G

297 (C/G)AA (C/G)TG A(T/A)G (A/G)TT A(G/T)C (G/C)TG GTG AAC C(G/T)G GGC 326  
 (G/C)TT (G/C)AC T(A/T)C (T/C)AA T(C/A)G (C/G)AC CAC TTG G(C/A)C CCG  
 Q/E L/V M/K I/V S/I V/L N N R/L G

327 (C/A)A(G/C) GA(T/A) GAA (T/G)TT (A/G) (C/T)G ATT (C/G)AG C(C/G)G GGC GAA 356  
 (G/T)T(C/G) CT(A/T) CTT (A/C)AA (T/C) (G/A)C TAA (G/C)TC G(G/C)C CCG CTT  
 Q/N/H/K D/E E F/V T/V/M/A I Q/E P/R G E

**FspI**

357 CGT ATT GCG CAG (A/C)TG (A/G)TT (T/A)TT G(T/C)G CCG GTG (G/C) (T/A)G 389  
 GCA TAA CGC GTC (T/G)AC (T/C)AA (A/T)AA C(A/G)C GGC CAC (C/G) (A/T)C  
 R I A Q M/L I/V F/I V/A P V V/Q/E/L

390 C(A/G)G G(C/T)G GAA (T/G)TT (A/G) (A/T)T (C/G) (T/A)G GTG GAA GA(T/A) 416  
 G(T/C)C C(G/A)C CTT (A/C)AA (T/C) (T/A)A (G/C) (A/T)C CAC CTT CT(A/T)  
 Q/R A/V E F/V N/V/I/D L/E/Q/V V E D/E

417 (T/G)TT TCT CAG ACC (G/C)A(T/G) CGT GGC GAA GGC GGC TTT GGC TCT A(G/C)C 458  
 (A/C)AA AGA GTC TGG (C/G)T(A/C) GCA CCG CTT CCG CCG AAA CCG AGA T(C/G)G  
 F/V S Q T D/Q/E/H R G E G G F G S S/T

**BamHI EcoRI**

459 GGC A(G/C)A (C/A)AG TAA TGA GGATCC GAATTC TT 487  
 CCG T(C/G)T (G/T)TC ATT ACT CCTAGG CTTAAG AA  
 G R/T Q/K \* \*

Figure 7

10 20 30 40 50 60 70  
TTGGTACCAAGCTTCATATGARMAAARTTRWTSTGAAAATTMWGCGTCYGCSTCATGSCMAAGAWTTWCC  
AACCATGGTTCGAAGTATACTYKTTTTYAAWASACTTTTAAKWCGCAGRCGSAGTACSGKTTCTWAAWGG

80 90 100 110 120 130 140  
GCTGCCGASCTATGCGACCYCTCACAGCKCAGGCCTGGATCTGCGTGCGKSCMTTRASRAWSCGSTGRAA  
CGACGGCTSGATACGCTGGRGAGTGTCGMGTCCGGACCTAGACGCACGMSGKAAYTSYTWSGCSACYTT

150 160 170 180 190 200 210  
MTTRMGCCGKKTGAWASGRYGCTGRITCCGACCGGTCTGATCMTTSAWATTSCGGAWGGTTMTGMGGSGC  
KAAYKCGGCMMACTWTSCYRCGACYAAGGCTGGCCAGACTAGKAASTWTAASGCCTWCCAAKACKCCSCG

220 230 240 250 260 270 280  
AGRTGCKGCCGCTAGCGGCCTGGSCCTGGAAMAKGGCWTARYGSTGCTGAACGCGSYGGGCMYGATCGA  
TCYACGMCGGCGCATCGCCGGACCSGACCTTTKTMCCGWATYRCSACGACTTGCGCSRCCCGKRCTAGCT

290 300 310 320 330 340 350  
TRSCGATTATCRGGGCSAASTGAWGRTTAKCSTGGTGAACCKGGGCMASGAWGAAKTTRYGATTSAAGCSG  
AYSGCTAATAGYCCCGSTTSACTWCYAATMGSACCACTTGMCCCGKTSCTWCTTMAAYRCTAASTCGSC

360 370 380 390 400 410 420  
GGCGAACGTATTGCGCAGMTGRTTWTGCGCCGGTGSWGCRCGGYGGAAKTTRWTSWGGTGGAAGAWKTTT  
CCGCTTGCTATAACGCGTCKACYAAWAACRCGGCCACSWCGYCCRCCTTMAAYWASWCCACCTTCTWMAAA

430 440 450 460 470 480  
CTCAGACCSAKCGTGGCGAAGGCGGCTTTGGCTCTASCGGCASAMAGTAATGAGGATCCGAATTCTT  
GAGTCTGGSTMGCACCGCTTCGCCGAAACCGAGATSGCCGTSTKTCATTACTCCTAGGCTTAAGAA

Figure 8.

14  
atg atc ctg gat g(t/c)t gac tac atc act gaa ga(a/c) ggc aaa ccg (g/a)tt atc cgt (c/a)t(g/c) ttc  
M I L D V/A D Y I T E E/D G K P V/I I R L/I/M F  
  
aaa aaa gag aac ggc (a/g)aa ttt aag (a/g)tt gag (c/t)at gat cgc a(a/c)c ttt cgt cca tac att tac gct  
K K E N G K/E F K I/V E H/Y D R N/T F R P Y I Y A  
  
ctg ctg a(g/a)a gat gat tct (a/c)ag att ga(g/t) gaa gtt a(g/a)a aaa atc act g(g/c)t gag cgc cat ggc aag att  
L L R/K D D S K/Q I E/D E V K/R K I T G/A E R H G  
K I  
  
214 215  
gtt cgt atc (a/g)tt gat g(t/c)g gaa aag gta (g/a)(g/a)g aag aaa ttt ctg ggc a(a/g)a cca atc (a/g)(a/c)g  
V R I I/V D V/A E K V G/E/R/K K K F L G K/R P I  
K/T/E/A  
  
gtg tgg a(g/a)a ctg tat (c/t)tc gaa cat cca caa gat gtt ccg a(t/c)t att cgc ga(g/t) aaa (g/a)tt cgc  
V W K/R L Y L/F E H P Q D V P T/A I R E/D K V/I R  
  
gaa cat (c/t)ct gca gtt (g/a)tt gac atc ttc gaa tac gat att cca ttt gca aag cgt tac ctc atc gac aaa  
E H P/S A V V/I D I F E Y D I P F A K R Y L I D K  
  
ggc ctg ata cca atg gag ggc ga(g/t) gaa gaa ctc aag (a/c)tc ctg gcg ttc gat ata gaa acc ctc tat  
G L I P M E G E/D E E L K I/L L A F D I E T L Y  
  
cac gaa ggc gaa gag ttt g(g/c)t aaa ggc cca att ata atg att agc tat gca gat gaa (a/g)a(a/c) gaa gca aag  
H E G E E F G/A K G P I I M I S Y A D E K/N/E/D E A  
K  
  
gtg att act tgg aaa aa(a/c) ata gat ctc cca tac gtt gag gtt gta tct tcc gag cgc gag atg att aag cgc  
V I T W K K/N I D L P Y V E V V S S E R E M I K R  
  
ttt ctc a(g/a)a (g/a)tt atc cgc gag aag gat ccg gac (g/a)tt atc (g/a)tt act tat aac ggc gac tct ttt  
F L R/k V/I I R E K D P D V/I I V/I T Y N G D S F  
  
gac (c/t)tc cca tat ctg g(t/c)g aaa cgc gca gaa aaa ctc ggt att aaa ctg (a/c)ct (a/c)tc ggc cgt gat ggt  
D F P Y L V/A K R A E K L G I K L T/P I/L G R D  
G  
  
tcc gag ccg aag atg cag cgt (a/c)tc ggc gat atg acc gct gta gaa (g/a)tt aag ggt cgt atc cat ttc gac  
S E P K M Q R I/L G D M T A V E V/I K G R I H F D  
  
ctg tat cat gta att (a/c)(c/g)c cgt act att aac ctc ccg act tac act ctc gag gct gta tat gaa gca att  
L Y H V I T/S/P/R R T I N L P T Y T L E A V Y E A I  
  
ttt ggt aag ccg aag gag aag gta tac gcc (g/c)at gag att gca (a/g)ag gcg tgg gaa (a/t)cc ggt (a/g)ag  
F G K P K E K V Y A D/H E I A K/E A W E T/S G K/E  
  
(a/g)(a/g)c ctc gag cgt gtt gca aaa tac tcc atg gaa gat gca aag g(t/c)g act tat gaa ctc ggc a(g/a)a gaa ttc  
N/G/D/S L E R V A K Y S M E D A K V/A T Y E L G R/K  
E F

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(c/t)tc cca atg gaa (a/g)(t/c)t cag ctc tct cgc ctg gtt ggc caa cca ctg tgg gat gtt tct cgt tct tcc  
L/F P M E I/T/V/A Q L S R L V G Q P L W D V S R S S

acc ggt aac ctc gta gag tgg t(t/a)t ctc ctg cgc aaa gcg tac gaa cgc aac gaa (g/c)tg gct ccg aac aag  
T G N L V E W F/Y L L R K A Y E R N E V/L A P N K

cca (t/g)(c/a)c gaa (c/g)(g/a)a gag tat (c/g)aa cgc cgt ctc cgc gag tct tac (a/g)ct ggt ggc t(t/a)t  
P S/Y/A/D E R/Q/G/E E Y Q/E R R L R E S Y T/A G G F/Y

ggt aaa gag cca gaa aag ggc ctc tgg gaa (a/g)(a/g)c (a/c)tc gtg t(c/a)c ctc gat ttt cgc (g/t)ct ctg  
V K E P E K G L W E N/S/D/G I/L V Y/S L D F R A/S L

tat ccg tct att atc att acc cac aac gtg tct ccg gat act ctc aac c(t/g)c gag ggc tgc a(g/a)a  
Y P S I I I T H N V S P D T L N L/R E G C K/R

(a/g)a(a/c) tat gat (g/a)tt gct ccg (c/g)aa gta ggc cac aag ttc tgc aag gac (a/t)tc ccg ggc ttt att  
K/N/E/D Y D V/I A P Q/E V G H K F C K D I/F P G F I

ccg tct ctc ctg (a/g)(a/g)g c(a/g)t ctg ctc ga(g/t) gaa cgc caa (a/g)ag att aag (a/c)(g/c)c aaa atg aag  
P S L L K/R/E/G R/H L L E/D E R Q K/E I K T/S/P/R K M K

g(a/c)g (a/t)cc (c/a)ag gat ccg att gaa aaa a(t/a)a (a/c)tg ctc gac tat cgc caa a(g/a)a gcg att aaa  
E/A T/S Q/K D P I E K I/K M/L L D Y R Q R/K A I K

(a/c)tc ctc gca aac tct t(a/t)t tac ggc tat tat ggc tat gca aaa gca cgc tgg tac tgt aag gag tgt gct  
L/I L A N S F/Y Y G Y Y G Y A K A R W Y C K E C A

gag tcc gtt act gct tgg ggt cgc (a/g)aa tac atc gag (c/t)tc gtg (t/c)gg aag gag ctc gaa gaa aag ttt ggc  
E S V T A W G R K/E Y I E L/F V W/R K E L E E K F G

ttt aaa gtt ctc tac att gac act gat ggt ctc tat gcg act att ccg ggt g(g/c)t (a/g)ag (c/t)ct gag  
F K V L Y I D T D G L Y A T I P G G/A E/K S/P E

1696

gaa att aag aaa aag gct ctc gaa ttt gtg aaa tac att aac (g/t)cg aag ctc ccg ggt ctc ctg gag ctc gaa  
E I K K K A L E F V K(D) Y I N A/S K L P G L L E L E

tat gaa ggc ttt tat (g/a)(t/a)g cgc ggc ttc ttc gtt acc aag aag a(g/a)a tat gcg (g/c)tg att gat gaa gaa  
Y E G F Y V/E/M/K R G F F V T K K R/K Y A V/L I D E E

ggc aaa (g/a)tt att act cgt ggt ctc gag att gtg cgc cgt gat tgg agc gaa att gcg aaa gaa act caa gct  
G K V/I I T R G L E I V R R D W S E I A K E T Q A

a(g/a)a gtt ctc gag (a/g)ct att ctc aaa cac ggc (g/a)ac gtt gaa gaa gct gtg a(g/a)a att gta aaa gaa gta  
R/K V L E T/A I L K H G D/N V E E A V R/K I V K E V

a(t/c)c (c/g)aa aag ctc (g/t)ct aa(a/c) tat gaa att ccg cca gag aag ctc g(t/c)g att tat gag cag att  
I/T Q/E K L A/S K/N Y E I P P E K L V/A I Y E Q I

act cgc ccg ctg cat gag tat aag gcg att ggt ccg cac gtg gct gtt gca aag a(g/a)a ctg gct gct a(g/a)a ggc gtg  
T R P L H E Y K A I G P H V A V A K R/K L A A K/R G V

aaa (g/a)tt a(g/a)a ccg ggt atg gta att ggc tac att gta ctc cgc ggc gat ggt ccg att agc aa(a/c) cgt gca  
K V/I R/K P G M V I G Y I V L R G D G P I S K/N R A

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att cta gct gag gaa t(t/a)c gat c(c/t)g a(g/a)a aag cac aag tat gac gca gaa tat tac att gag aac cag gtg  
I L A E E F/Y D P/L K/R K H K Y D A E Y Y I E N Q V  
2272 2273  
ctc ccg gcg gta ctc cgt att ctg gag g(g/c)t ttt ggc tac cgt aag gaa gac ctc cgc t(a/g)(c/g) caa aag  
L P A V L R I L E G/A F G Y R K E D L R Y/W/\*C Q K  
act a(g/a)a cag (g/a)(t/c)t ggc ctc act (g/t)ct tgg ctc aac att aaa aaa tcc ggt acc cac tag tgc tag cat gac  
T K/R Q V/A/I/T G L T A/S W L N I K K S G T H \*

Figure 9. A comparison of the polymerase to 3' to 5' exonuclease activity

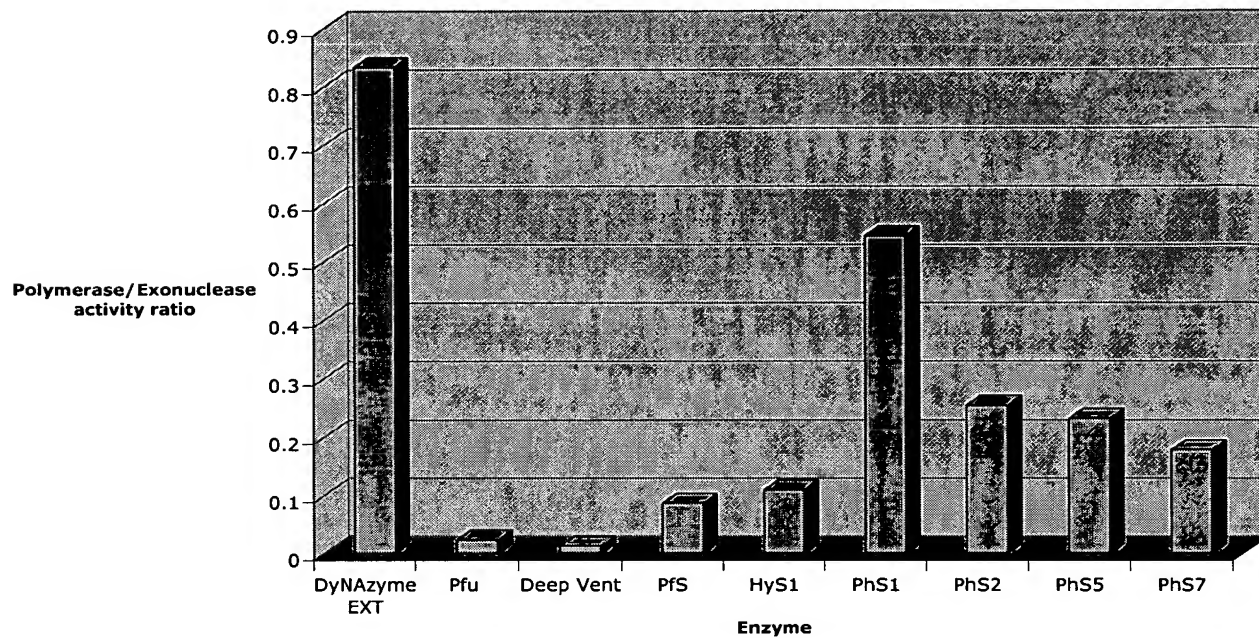




FIGURE 10

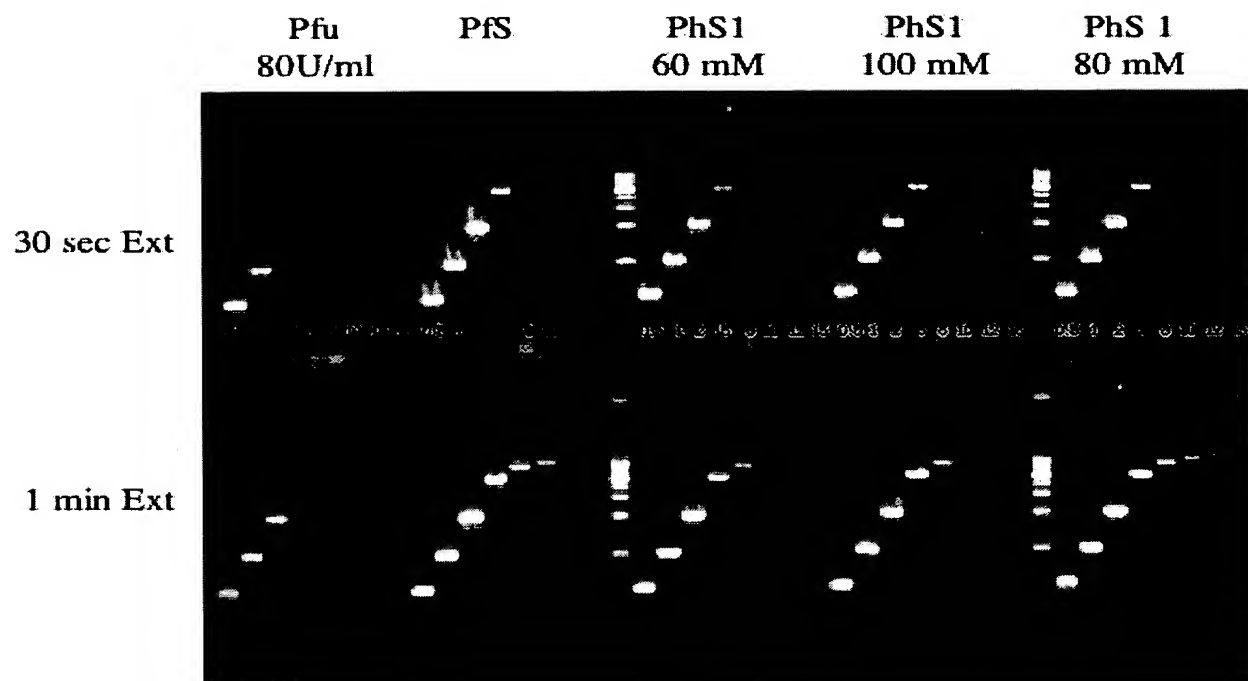
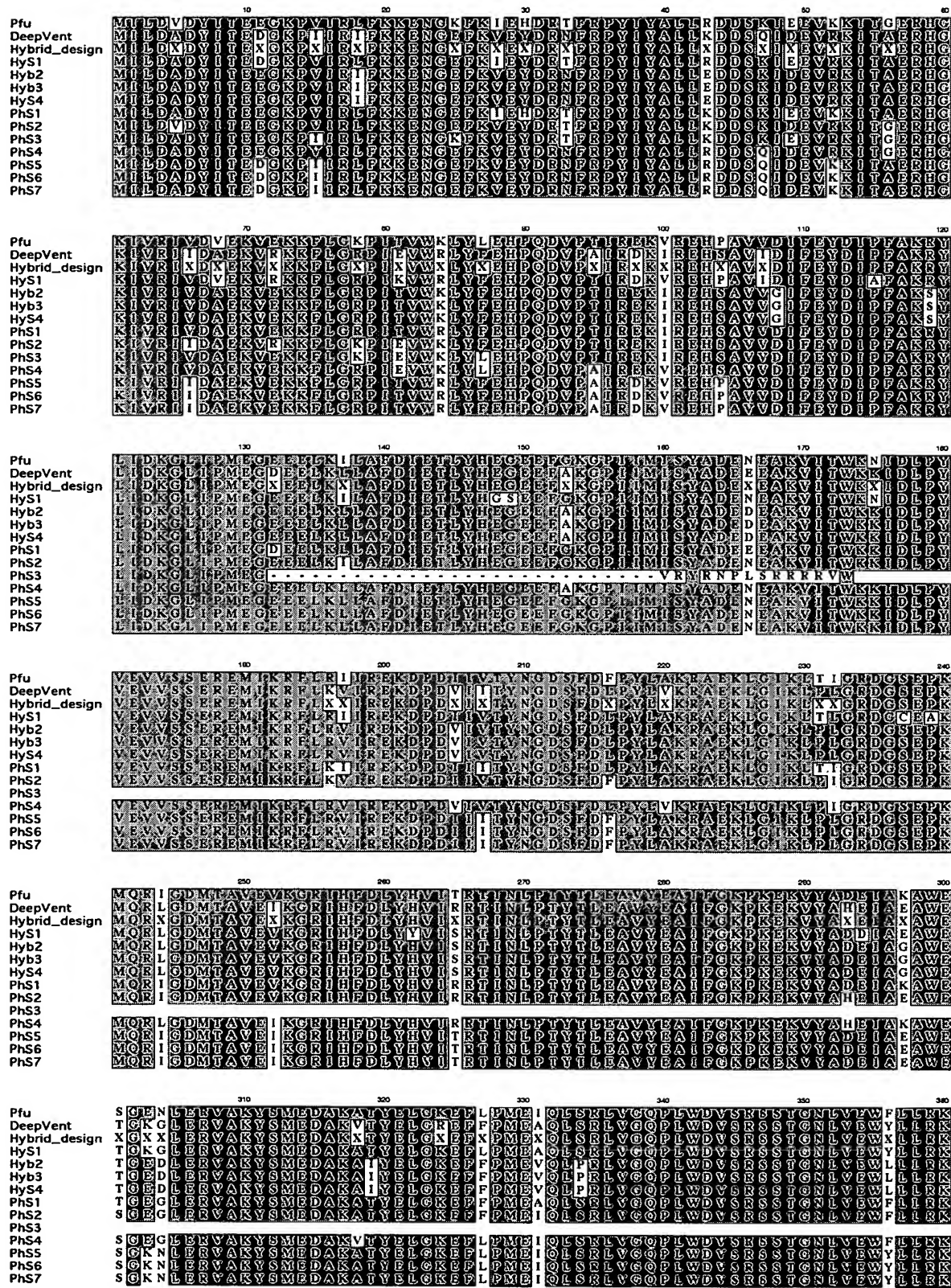


Figure 11





	730	740	750	760	770	780
Pfu	Y D P	K K H K Y D A E Y Y I E N Q V L P A V L R I L E A	G F C Y R K E D L R W O K T K Q	V G L T S	W L N I K K S G T H N C	
DeepVent	F D L R	K K H K Y D A E Y Y I E N Q V L P A V L R I L E A	G F C Y R K E D L R W O K T K Q	V G L T S	W L N I K K S G T H N C	
Hybrid_design	X D X X	K K H K Y D A E Y Y I E N Q V L P A V L R I L E A	G F C Y R K E D L R W O K T K Q	V G L T S	W L N I K K S G T H N C	
HyS1	F D L R	K K H K Y D A E Y Y I E N Q V L P A V L R I L E A	G F C Y R K E D L R W O K T K Q	V G L T S	W L N I K K S G T H N C	
Hyb2	F D L R	K K H K Y D A E Y Y I E N Q V L P A V L R I L E A	G F C Y R K E D L R W O K T K Q	V G L T S	W L N I K K S G T H N C	
Hyb3	F D L R	K K H K Y D A E Y Y I E N Q V L P A V L R I L E A	G F C Y R K E D L R W O K T K Q	V G L T S	W L N I K K S G T H N C	
HyS4	F D L R	K K H K Y D A E Y Y I E N Q V L P A V L R I L E A	G F C Y R K E D L R W O K T K Q	V G L T S	W L N I K K S G T H N C	
PhS1	Y D P	K K H K Y D A E Y Y I E N Q V L P A V L R I L E A	G F C Y R K E D L R W O K T K Q	V G L T S	W L N I K K S G T H N C	
PhS2	Y D L	K K H K Y D A E Y Y I E N Q V L P A V L R I L E A	G F C Y R K E D L R W O K T K Q	V G L T S	W L N I K K S G T H N C	
PhS3						
PhS4	Y D P	K K H K Y D A E Y Y I E N Q V L P A V L R I L E A	G F C Y R K E D L R W O K T K Q	V G L T S	W L N I K K S G T H N C	
PhS5	Y D L	K K H K Y D A E Y Y I E N Q V L P A V L R I L E A	G F C Y R K E D L R W O K T K Q	V G L T S	W L N I K K S G T H N C	
PhS6						
PhS7	Y D L	K K H K Y D A E Y Y I E N Q V L P A V L R I L E A	G F C Y R K E D L R W O K T K Q	V G L T S	W L N I K K S G T H N C	

	790	800	810	820	830	840
Pfu						
DeepVent						
Hybrid_design	N H D					
HyS1	G A T V K F R Y K G E E K E V D I S K I K K Y W R V G K M I S F T Y D E C G G K T G R G A V S E K D A P K E L L Q M L E					
Hyb2						
Hyb3						
HyS4	G A T V K F R Y K G E E K E V D I S K I K K Y W R V G K M I S F T Y D E C G G K T G R G A V S E K D A P K E L L Q M L E					
PhS1	G A T V K F R Y K G E E K E V D I S K I K K Y W R V G K M I S F T Y D E C G G K T G R G A V S E K D A P K E L L Q M L E					
PhS2	G A T V K F R Y K G E E K E V D I S K I K K Y W R V G K M I S F T Y D E C G G K T G R G A V S E K D A P K E L L Q M L E					
PhS3						
PhS4	G A T V K F R Y K G E E K E V D I S K I K K Y W R V G K M I S F T Y D E C G G K T G R G A V S E K D A P K E L L Q M L E					
PhS5	G A T V K F R Y K G E E K E V D I S K I K K Y W R V G K M I S F T Y D E C G G K T G R G A V S E K D A P K E L L Q M L E					
PhS6						
PhS7	G A T V K F R Y K G E E K E V D I S K I K K Y W R V G K M I S F T Y D E C G G K T G R G A V S E K D A P K E L L Q M L E					

	850	860	870	880	890	900
Pfu						
DeepVent						
Hybrid_design	K Q K K N					
HyS1						
Hyb2						
Hyb3						
HyS4	K Q K K N					
PhS1	K Q K K N					
PhS2	K Q K K N					
PhS3						
PhS4	K Q K K N					
PhS5	K Q K K N					
PhS6						
PhS7	K Q K K					